filed August 22, 1997. The Examiner also indicated that a translation of the priority document has not been provided.

Applicants thank the Examiner for clarifying that a translation of the priority document DE 197 36 691.0 (filed August 22, 1997) is not requested or required at this time.

REJECTIONS UNDER 35 U.S.C. § 103

The PTO rejects claims 21-22, 24-28, 32, 36-37, 41-45, 49-51, and 54-59 under 35 U.S.C. § 103, asserting the claims are obvious over Jung et al. (*Eur. J. Clin. Chem. Clin. Biochem.* 35:3-10 (1997)) and Rimm et al. (U.S. Pat. No. 6,197,523 (March, 2001)) or Ts'o et al. (U.S. Pat. No. 5,962,237 (October, 1999)) in view of Hoon et al. (U.S. Pat. No. 6,057,105 (May, 2000)). The Examiner bases this rejection on the interpretation that the claims encompass detection of a nucleic acid in both an enriched sample and in a sample not enriched for cancer cells. More specifically, the Examiner alleges that a person having ordinary skill in the art would have been motivated to combine the teachings of Jung et al. (detection of single metastatic cancer cells by RT-PCR in a peripheral blood sample) and Rimm et al. (performing two different types of assays using the same cell sample), or Ts'o et al. (removing non-rare cells from a fluid to increase the number of cancer cells in a sample), in view of Hoon et al. (detection of metastasis of melanoma and breast cancer using more than one cancer cell marker), to obtain Applicants' invention.

Applicants note that on page 7 of the Office Action, the Action refers to the method of Ditkoff. Because Ditkoff was not cited in the introductory paragraph of this rejection, Applicants assume that the Action intended to cite Jung et al. rather than Ditkoff, and have responded accordingly.

Applicants respectfully traverse these grounds for rejection and submit that the documents cited by the Action, alone or in combination, fail to teach or suggest the subject matter of the instant claims. Applicants' invention is directed in pertinent part to a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject. The method comprises (a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a

subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from the body fluid according to a method for isolating cancer cells from non-cancer cells. In certain embodiments, the method further comprises detecting an absence or presence of at least one first nucleic acid selected from a first cancer-specific nucleic acid or a first cancer-associated nucleic acid in the first fraction; and detecting in the second fraction, an absence or presence of at least one second nucleic acid selected from a second cancer-specific nucleic acid or a second cancer-associated nucleic acid; and detecting in at least one non-cancer cell from the subject an absence or presence of the at least one second nucleic acid, wherein the presence of the first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in the second fraction relative to the presence or absence of the second nucleic acid in a non-cancer cell indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

In certain other embodiments, the method comprises detecting in the first fraction (not subjected to a method for isolating cancer cells from non-cancer cells), at least one first cancer-specific nucleic acid, and detecting in the second fraction (subjected to a method for isolating cancer cells from non-cancer cells) an absence or presence of at least one second cancer-specific nucleic acid, and detecting at least one cancer-associated nucleic acid in the first fraction or in the second fraction.

Applicants submit that a person having ordinary skill in the art would not have been motivated by the cited publications alone or in combination to arrive at the present invention with any reasonable expectation of success. Accordingly, and for the reasons discussed herein, Applicants respectfully submit that the PTO has not established a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 1339, 1341-42, 41 U.S.P.Q.2d 1451 (Fed. Cir. 1997) (PTO has the burden of showing a *prima facie* case of obviousness.)). The PTO must show (1) that the combined references teach or suggest all claim limitations; (2) that the references provide some teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the claimed invention; and (3) that the combined teachings of the references indicate that by combining the references, a person having ordinary skill in the art will achieve the claimed

invention with a reasonable expectation of success. When rejection of claims depends upon a combination of prior art references, a teaching, motivation, or suggestion to combine the references must exist. (*See In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998).

Applicants submit that Jung et al. and Rimm et al. or T'so et al. in view of Hoon et al., alone or in combination, fail to teach or suggest all limitations of the claimed method. Each document fails to teach or suggest a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell, comprising dividing a plurality of cells into a first fraction that has not been subjected to a method for isolating cancer cells from non-cancer cells, and a second fraction that has been subjected to such a method. Each of the cited publications also fails to teach or suggest detecting an absence or presence of at least one first cancer-specific or cancer-associated nucleic acid in the first fraction, and detecting an absence or presence of at least one second cancer-specific or cancer-associated nucleic acid in the second fraction. Also, none of the cited documents teach or suggest detecting an absence or presence of the second nucleic acid in a non-cancer cell from the subject.

Jung et al. teach a RT-PCR method for detecting a single polynucleotide encoding a tumor related antigen solely in an unfractionated sample such as peripheral blood. Jung et al. fail to teach or suggest detecting a first nucleic acid in unfractionated blood <u>and</u> detecting a second nucleic acid in a second fraction that has been enriched for cancer cells. Moreover, Jung et al. suggest that "no enrichment of nucleated cells [including cancer cells] is necessary," thus teaching away from the claimed method comprising detecting cancer-specific or cancer-associated nucleic acids in both fractionated and unfractionated cells.

Rimm et al. disclose a method for separating cancer cells of epithelial origin and hematologic progenitor cells from whole blood on the basis of differences in cell density, followed by visual morphometric analysis and epitopic analysis, that is, detection of an antigen expressed on the cell using an antibody that specifically binds to the antigen. Rimm et al. suggest that to verify morphometric analysis, other methods such as RT-PCR may be used on cells that are separated by the disclosed method. However, Rimm et al. do not suggest the desirability of combining the method taught therein with any other method known in the art for detecting a first cancer related nucleic acid in unfractionated cells and detecting a second cancer

related nucleic acid in fractionated cells. Furthermore, and in contradiction to the teachings of Jung et al., Rimm et al. teach that detection of nucleic acids by RT-PCR methods is not a practical technique for detecting circulating cancer cells (*see* Rimm et al., column 2, lines 20-38). Therefore, Rimm et al. also teach away from detecting a disseminated or micrometastasized cancer cell in a body fluid using the claimed method. Moreover, in view of the contradictory teachings of Rimm et al. and Jung et al., Applicants submit that the PTO has not established that suggest that a person having ordinary skill in the art could combine the teachings found therein these publications and reasonably expect to achieve Applicants' invention successfully.

Ts'o et al. teach a method for enriching rare cells from a body fluid and then subjecting the enriched cells to methods for detecting cancer cells. Ts'o et al., however, do not teach or suggest the desirability of determining the presence of disseminated or micrometastasized cells by detecting a cancer-specific or cancer-associated nucleic acid in a fraction not subjected to a method for isolating cancer cells from non-cancer cells <u>and</u> in a second fraction enriched for cancer cells.

Hoon et al. do not remedy the deficiencies of Jung et al., Rimm et al., or Ts'o et al. Hoon et al. teach a method for detecting one or more markers of a melanoma or breast cancer cell in cells from a subject, and detecting the presence of the same markers in cells from persons who do not have the disease. Hoon et al. do not teach or suggest detecting the markers in a first unfractionated sample of a body fluid <u>and</u> in a second fraction enriched for cancer cells. Hoon et al. also fail to teach or suggest the desirability of combining the method disclosed therein with any other method that includes a step for detecting the melanoma or breast cancer cell markers in a non-cancer cell isolated from the same subject.

Additionally, Applicants submit that the Action fails to point to any teaching, suggestion, or motivation for a person having ordinary skill in the art to modify or combine the cited documents to achieve the claimed invention with a reasonable expectation of success. Furthermore, none of the cited art suggests the desirability of combining any of the disclosures described therein and then modifying the teachings to achieve Applicants' invention. Each of the cited documents discloses a method using either a body fluid that has not been treated in some manner to enrich cancer cells <u>or</u> a method using a body fluid that has been subjected to a method to enrich and select cancer cells. None of the cited documents teaches using a fractionated <u>and</u>

an unfractionated sample, and none suggest the desirability of using two such fractions in a method for detecting a cancer cell. Moreover, and as discussed above, the contradictory teachings of Jung et al. and Rimm et al. suggests a lack of desirability to analyze cancer-related nucleic acids in both a fractionated and an unfractionated cell sample. Furthermore, even though a person having ordinary skill in the art could analyze an unfractionated sample as disclosed in Jung et al. or Hoon et al., and could analyze a fractionated sample enriched for cancer cells, such as by the method of Rimm et al. or Ts'o et al., such a combination does not render the claimed method obvious without a suggestion in the art of the desirability to make the combination. Obviousness may not be established by combining teachings of the cited art absent some teaching that supports the combination. (*See In re Fritch*, 922 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992) (finding that the mere fact that modification of the prior art may reflect features of the claimed invention does not make the invention obvious unless desirability of the modification is suggested by the prior art).

Applicants therefore respectfully submit that the subject matter of the claims is nonobvious as required under 35 U.S.C. § 103 and respectfully request that the rejection of the claims be withdrawn.

The PTO also rejects claims 21-22, 27-37, 41-43, 45-49, 51, and 56 under 35 U.S.C. § 103, alleging that the claims are obvious over Schmitz et al. (U.S. Patent No. 6,190,870 (February, 2001)) in view of Popescu et al. (*Cancer Genet. Cytogenet.* 93:10-21 (1997)) or Torczynski et al. (U.S. Patent No. 5,589,579 (December 1996)) and in further view of Hoon et al. (U.S. Patent No. 6,057,105 (May, 2000)). The Examiner bases the rejection on the interpretation that the claims encompass a method in which both fractions may be enriched for cancer cells. In particular, the Examiner asserts that it would have been obvious to achieve Applicants' invention by combining the teachings of Schmitz et al., describing a tumor cell enrichment method, with the disclosure of detection methods in Popescu et al. (identifying chromosomal translocations using fluorescent in situ hybridization, or FISH) or Torczynski et al. (detecting cancer at the mRNA level using FISH), and with the teachings of Hoon et al. regarding detecting metastasis of melanoma and breast cancer using more than one cancer cell marker. The Examiner further alleges that with respect to claims 41-43, an ordinary artisan would be motivated to use the same

first and second cancer-specific nucleic acids or different first and second nucleic acids, and would have been motivated to use the same nucleic acids in both steps for detecting certain point mutations that were specific for certain cancers or that indicated a prognosis of cancer.

Applicants respectfully traverse the basis for the rejection and submit that the documents cited by the Action, alone or in combination, fail to teach or suggest the subject matter of the instant claims. For reasons discussed above with respect to the documents cited above in the preceding rejection, each of Schmitz et al., Popescu et al., Torczynski et al., and Hoon et al. also fail to teach or suggest a method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell, comprising dividing a plurality of cells into a first fraction that has <u>not</u> been subjected to a method for isolating cancer cells from non-cancer cells <u>and</u> a second fraction that has been subjected to such a method. Each of the cited publications also fails to teach or suggest detecting at least one first cancer-specific or cancer-associated nucleic acid in the first fraction, and detecting at least one second cancer-specific or cancer-associated nucleic acid in the second fraction. Also, none of the cited documents teach or suggest detecting the second nucleic acid in a non-cancer cell from the subject.

As the PTO concedes, Schmitz et al. fail to teach or suggest identifying a first and a second cancer-specific or cancer-associated nucleic acid in two separate fractions. Schmitz et al. teach a method for enriching carcinoma cells and analyzing <u>only</u> the enriched fraction to quantify the number of cancer cells present in the sample. Applicants submit that Popescu et al., Torczynski et al., and Hoon et al. all fail to remedy the deficiencies of Schmitz et al. Popescu et al., merely provide a general review of several cytogenetic methods, including FISH, for detecting chromosomal abnormalities in cancer cells. Popescu et al. fail to suggest any desirability of using FISH for analyzing unfractionated cells and a fraction enriched for cancer cells, or for analyzing disseminated or micrometastasized cells. Torczynski et al. disclose a method for diagnosing lung cancer using several markers, including those well known in the art such as CEA, NCA, and the ras and myc families of oncogenes. Torczynski et al. fail, however, to suggest any desirability of combining detection of these markers with carcinoma cells enriched according to Schmitz et al., or with any other method known in the art to achieve Applicants' invention. As discussed above, Hoon et al. teach a method for detecting, in cells from a subject,

one or more markers of a melanoma or breast cancer cell. Hoon et al. fail to teach or suggest detecting the markers in a first unfractionated sample of a body fluid <u>and</u> in a second fraction enriched for cancer cells. Hoon et al. also fail to teach or suggest detecting the melanoma or breast cancer cell markers in a non-cancer cell isolated from the same subject.

Applicants submit that any combination of Schmitz et al., Popescu et al., Torczynski et al., and Hoon et al. fails to teach or suggest all limitations of Applicants' invention, particularly detection of cancer-specific or cancer-associated nucleic acids in both fractionated and unfractionated cells from a body fluid. Furthermore, none of the cited documents teach or suggest the requisite modifications of any of the disclosed techniques that would be required to achieve the claimed method; nor do any of the cited documents teach or suggest the desirability of making such modifications. Applicants submit that to so modify the art could only be accomplished using impermissible hindsight in view of the instant application. Applicants therefore submit that the claimed invention is nonobvious as required under 35 U.S.C. § 103 and respectfully request that the rejection of the claims be withdrawn.

The PTO rejects claims 38-39 and 52-53 under 35 U.S.C. § 103 for allegedly being obvious over Mitsuhashi (U.S. Patent No. 5,976,797 (November 1999)) in view of Jung et al. and Rimm et al. or Ts'o et al. in view of Hoon et al., as applied above in the first basis for rejection. More specifically, the Action asserts that it would have been obvious for a person having ordinary skill in the art to obtain Applicants' invention by modifying the method disclosed by Mitsuhashi regarding detection of cytotoxic effects of anticancer compounds by measuring mRNA expression, in view of the disclosures in Jung et al., Rimm et al., or Ts'o et al. as asserted above for detecting cancer cells in either enriched or unenriched cell fractions. The Action further alleges that an ordinary artisan would have been motivated to analyze more than one mRNA for reasons of specificity and reliability as provided by Hoon et al.

Applicants note that on page 12 of the Office Action, the Action states that "[n]either Schimitz [*sic*], Popescu, Torczynski nor Hoon teach analyzing and identifying an anticancer therapy." Applicants assume that this recitation of publications different than those cited on page 11 of the Action was an inadvertent error and have responded to this rejection on

the basis of the disclosures in Mitsuhashi, in view of Jung et al. and Rimm et al. or Ts'o et al., in view of Hoon et al.

Applicants respectfully traverse these grounds for rejection and submit that the subject matter of claims 38-39 and 52-53 is nonobvious. Applicants' invention is directed in pertinent part to a method for identifying an anticancer therapy, comprising detecting, before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, (i) in a plurality of cells obtained from a body fluid of the subject, the absence or presence of at least one first cancer-specific or cancer-associated nucleic acid; and (ii) the absence or presence in at least one cancer cell removed from the plurality of cells, at least one second cancer-specific or cancer-associated nucleic acid; and (iii) in at least one non-cancer cell from the subject, the absence or presence of the nucleic acids from step (i) and step (ii), wherein the first and second cancer-specific nucleic acids are different, and wherein the first and second cancer-associated nucleic acids are different. In another embodiment, the subject matter is directed in pertinent part to a method for identifying an anticancer agent, comprising detecting (i) in a plurality of cells an absence or presence of at least one first cancer-specific or cancer-associated nucleic acid, and (ii) in at least one cancer cell removed from the plurality of cells, the absence or presence of at least one second cancer-specific or cancer-associated nucleic acid, and (iii) in at least one non-cancer cell from the plurality of cells, the absence or presence of the nucleic acids from step (i) and step (ii), before and after contacting a candidate anticancer agent with the plurality of cells.

Applicants respectfully submit that Mitsuhashi alone or in combination with Jung et al., Rimm et al., Ts'o et al., or Hoon et al. fail to teach or suggest the claimed invention and further submit that none of these documents alone or in combination would have motivated a person having ordinary skill in the art to obtain Applicants' invention with a reasonable expectation of success. Applicants submit that none of the other cited documents in combination with Mitsuhashi teach or suggest all the limitations of the claimed method. As the PTO concedes, Jung et al., Rimm et al., Ts'o et al., and Hoon et al. all fail to teach or suggest analyzing an anticancer therapy or identifying an anticancer therapy by detecting the presence of first and second nucleic acids before and after contacting a candidate anticancer agent with cells. Mitsuhashi discloses a method for detecting the level of total mRNA isolated from cells before

and after the cells are exposed to a cytotoxic agent. Mitsuhashi also teaches that the level of a specific mRNA may be measured and compared with the total mRNA from the cells. Mitsuhashi fails, however, to teach or suggest detecting at least one first cancer-specific or cancer-associated nucleic acid in a plurality of unfractionated cells and fails to teach or suggest detecting a second cancer-specific or cancer-associated nucleic acid in a fraction enriched for cancer cells. Mitsuhashi further fails to teach or suggest detecting a cancer-related nucleic acid in non-cancer cells from a biological sample.

As discussed in detail above, Jung et al., Rimm et al., Ts'o et al., and Hoon et al. all fail to teach or suggest detecting one or more cancer-related nucleic acids in both a fractionated and an unfractionated sample. Furthermore, all the cited publications fail to teach or suggest detecting the cancer-related nucleic acid in a non-cancer cell. Briefly, Jung et al. teach a RT-PCR method for detecting a single polynucleotide encoding a tumor related antigen solely in an unfractionated sample such as peripheral blood. Rimm et al. disclose a cell enrichment method and a morphometric analytical technique to detect cancer cells, and suggest that to verify detection of a cancer cell, other methods such as RT-PCR may be used only with cells that are separated by the enrichment technique. Ts'o et al. teach a method for enriching rare cells from a body fluid and then subjecting <u>only</u> the enriched cells to methods for detecting cancer cells. Hoon et al. teach a method for detecting one or more markers of melanoma or breast cancer cells. Hoon et al., however, do not teach or suggest detecting the markers in a first unfractionated sample from a subject <u>and</u> in a second fraction enriched for cancer cells.

Applicants submit that Mitsuhashi does not teach or suggest the desirability of combining the method disclosed therein for identifying cytotoxic agents with any other method in the art for detecting disseminated or micrometastasized cells in an unfractionated sample and with any other method for detecting disseminated or micrometastasized cells in a sample enriched for cancer cells. Each of Jung et al., Rimm et al., Ts'o et al., and Hoon et al. fail to teach or suggest the desirability of combining the methods taught therein for enriching cancer cells and/or detecting cancer cells with any method for analyzing an anticancer therapy or identifying an anticancer therapy. Applicants further submit that none of the cited documents provides any motivation or suggestion of the desirability of combining the teachings therein and then making the requisite modifications to the disclosures to achieve Applicants' invention.

Also, and as discussed in detail above, the contradictory teachings of Jung et al. and Rimm et al. each teach away from Applicants' invention and thereby remove any motivation for a person having ordinary skill in the art to combine the teachings of the cited publications to achieve Applicants' invention with any reasonable expectation of success. (*See In re Fritch*, 922 F.2d at 1266) ("Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching supporting the combination.") (quoting *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 U.S.P.Q. 929 (Fed. Cir. 1984)). Applicants submit that only by using impermissible hindsight and applying knowledge of the presently claimed method, can the PTO allege that Applicants' invention would have been obvious to a person having ordinary skill in the art at the time the invention was made.

Applicants respectfully submit that a *prima facie* case of obviousness has not been set forth by the PTO, and submit that Applicants' invention is nonobvious as required under 35 U.S.C. § 103. Applicants therefore request that the rejection of the claims be withdrawn.

All claims remaining in the application are now allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Michael Giesing et al.

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 32, 38-39, 41-43, 45-46, 49, 52-53 have been amended and new claim 60 has been added as follows:

32. (Twice Amended) The method of ~~any one of~~ either claim~~s~~ 41 or claim 43 wherein at least one nucleic acid selected from the group consisting of ~~a~~ (i) a first cancer-associated nucleic acid, ~~and~~ (ii) a second cancer-associated nucleic acid, and (iii) a cancer-associated nucleic acid that is present in at least one cancer cell in the second fraction comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nucleic acid thereto.

38. (Amended) A method for identifying an anticancer therapy, comprising:

(a) detecting, before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell,

(i) in a plurality of cells obtained from a body fluid of the subject, an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell; ~~and~~

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid~~,~~; and

(iii) in at least one non-cancer cell from the subject, the absence or presence of said nucleic acids from step (i) and step (ii),

wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after administering the candidate anticancer therapy, a decreased presence of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell, and therefrom identifying an anticancer therapy.

39. <u>(Amended)</u> A method for identifying an anticancer agent, comprising:

(a) detecting in at least one cell, before and after contacting a candidate anticancer agent with a plurality of cells known to include or suspected of including a disseminated cancer cell or a micrometastasized cancer cell,

(i) an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, and

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, <u>and</u>

<u>(iii) in at least one non-cancer cell from said plurality of cells, the absence or presence of said nucleic acids from step (i) and step (ii),</u>

wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after contacting the candidate anticancer therapy with the cells, a decreased presence of any one or more of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in ~~a~~ <u>the</u> non-cancer cell, and therefrom identifying an anticancer agent.

41. <u>(Amended)</u> A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and <u>wherein the first fraction has not been</u>

subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid; ~~and~~

(c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid~~,~~; and

(d) detecting in at least one non-cancer cell from the subject an absence or presence of the at least one second nucleic acid that is detected in step (c),

wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic acid in said second fraction relative to the presence or absence of said second nucleic acid in ~~a~~ the non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

42. (Amended) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid; ~~and~~

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid~~,~~; and

(d) detecting in at least one non-cancer cell from the subject an absence or presence of the second cancer-specific nucleic acid that is detected in step (c),

wherein said first and second cancer-specific nucleic acids are different, wherein the presence of said first cancer-specific nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

43. (Amended) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid;

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid; ~~and~~

(d) detecting an absence or presence of at least one cancer-associated nucleic acid in at least one sample selected from the group consisting of (i) the first fraction and (ii) the second fraction;~~,~~ and

(e) detecting in at least one non-cancer cell from the subject an absence or presence of said second cancer-specific nucleic acid detected in step (c) and said cancer-associated nucleic acid detected in step (d),

wherein the presence of said first cancer-specific nucleic acid and of said cancer-associated nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid and of said ~~second~~ cancer-associated nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid and of said ~~second~~ cancer-associated nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

45. (Amended) The method of ~~any one of~~ claims 41-~~43~~ wherein a nucleic acid selected from the group consisting of (i) a first cancer-associated nucleic acid and (ii) a second cancer-associated nucleic acid, said nucleic acid ~~comprises~~ comprising a metastasis-associated gene, and wherein the presence of said first cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.

46. (Amended) The method of either claim 45 or claim 60 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

49. (Amended) The method of either claim 45 or claim 60 wherein the nucleic acid is selected from the group consisting of DNA and RNA.

52. (Amended) The method according to any one of claims 41-42 wherein steps (a) - (~~c~~d) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

53. (Amended) The method according to claim 43 wherein steps (a) - (~~d~~e) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

60. (New) The method of claim 43 wherein the cancer-associated nucleic acid comprises a metastasis-associated gene, and wherein the presence of the cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of the cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of the cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.

Appendix

Currently Pending Claims, Pending Entry of the Present Amendments

21. The method of claim 43 wherein the first and second cancer-specific nucleic acids are the same.

22. The method of claim 43 wherein the first and second cancer-specific nucleic acids are different.

24. The method of any one of claims 54-56 wherein the RNA comprises mRNA.

25. The method of claim 24 wherein the mRNA is not expressed in the non-cancer cell.

26. The method of claim 25 wherein the mRNA comprises all or a portion of a transcript of a gene selected from the group consisting of a CEA gene, a CK20 gene, a MUC1 gene, a tyrosinase gene and a MAGE3 gene.

27. The method of any one of claims 54-56 wherein the DNA that is detected comprises genomic DNA selected from the group consisting of genomic DNA comprising a genomic mutation, genomic DNA comprising a gene that has undergone amplification, genomic DNA comprising a gene that has undergone loss of heterozygosity, genomic DNA comprising a translocated gene and genomic DNA comprising a gene polymorphism.

28. The method of any one of claims 54-56 wherein at least one nucleic acid that is detected comprises DNA, said DNA comprising genomic DNA selected from the group consisting of (i) the second cancer-specific nucleic acid and (ii) a cancer-associated nucleic acid that is present in at least one cancer cell in the second fraction.

29. The method of any one of claims 54-56 wherein the DNA is genomic DNA that comprises all or a portion of an oncogene.

30. The method of any one of claims 54-56 wherein the DNA is genomic DNA that comprises all or a portion of a tumor suppressor gene.

32. (Amended) The method of either claim 41 or claim 43 wherein at least one nucleic acid selected from the group consisting of (i) a first cancer-associated nucleic acid, (ii) a second cancer-associated nucleic acid, and (iii) a cancer-associated nucleic acid that is present in at least one cancer cell in the second fraction comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nucleic acid thereto.

33. The method of claim 32 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

34. The method of claim 33 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

35. The method of claim 33 wherein the adhesion factor is an adherin.

36. The method of claim 24 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

37. The method of any one of claims 41-43 wherein the cancer cell is removed from the body fluid by a method selected from the group consisting of microfiltration, density gradient centrifugation and antigen-specific immunoadsorption.

38. (Amended) A method for identifying an anticancer therapy, comprising:

(a) detecting, before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell,

(i) in a plurality of cells obtained from a body fluid of the subject, an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell;

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid; and

(iii) in at least one non-cancer cell from the subject, the absence or presence of said nucleic acids from step (i) and step (ii),

wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after administering the candidate anticancer therapy, a decreased presence of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell, and therefrom identifying an anticancer therapy.

39. (Amended) A method for identifying an anticancer agent, comprising:

(a) detecting in at least one cell, before and after contacting a candidate anticancer agent with a plurality of cells known to include or suspected of including a disseminated cancer cell or a micrometastasized cancer cell,

(i) an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, and

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, and

(iii) in at least one non-cancer cell from said plurality of cells, the absence or presence of said nucleic acids from step (i) and step (ii),

wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after contacting the candidate anticancer therapy with the cells, a decreased presence of any one or more of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in the non-cancer cell, and therefrom identifying an anticancer agent.

41. (Amended) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid;

(c) detecting, in the second fraction, an absence or presence of at least one second nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid; and

(d) detecting in at least one non-cancer cell from the subject an absence or presence of the at least one second nucleic acid that is detected in step (c),

wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different, wherein the presence of said first nucleic acid in the first fraction and an increased or decreased presence of the second nucleic

acid in said second fraction relative to the presence or absence of said second nucleic acid in the non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

42. (Amended) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid;

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid; and

(d) detecting in at least one non-cancer cell from the subject an absence or presence of the second cancer-specific nucleic acid that is detected in step (c),

wherein said first and second cancer-specific nucleic acids are different, wherein the presence of said first cancer-specific nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

43. (Amended) A method for determining an increased risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject, comprising:

(a) dividing a plurality of cells into at least a first fraction and a second fraction, wherein each of the fractions comprises at least one cell and wherein the plurality of cells is from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, and wherein the first fraction has not been subjected to a method for isolating cancer cells from non-cancer cells, and wherein the second fraction comprises at least one cell that has been removed from said body fluid according to a method for isolating cancer cells from non-cancer cells;

(b) detecting in the first fraction an absence or presence of at least one first cancer-specific nucleic acid;

(c) detecting in the second fraction an absence or presence of at least one second cancer-specific nucleic acid;

(d) detecting an absence or presence of at least one cancer-associated nucleic acid in at least one sample selected from the group consisting of (i) the first fraction and (ii) the second fraction; and

(e) detecting in at least one non-cancer cell from the subject an absence or presence of said second cancer-specific nucleic acid detected in step (c) and said cancer-associated nucleic acid detected in step (d),

wherein the presence of said first cancer-specific nucleic acid and of said cancer-associated nucleic acid in said first fraction and an increased or decreased presence of said second cancer-specific nucleic acid and of said cancer-associated nucleic acid in said second fraction relative to the presence or absence of said second cancer-specific nucleic acid and of said cancer-associated nucleic acid in a non-cancer cell from the subject indicate an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

44. The method of any one of claims 41-43 wherein a nucleic acid selected from the group consisting of (i) a first cancer-specific nucleic acid and (ii) a first cancer-associated nucleic acid comprises an organotypical gene, and wherein the presence of at least one of said first nucleic acids comprising an organotypical gene indicates the type of malignant disease from which the cancer cell is derived.

45. (Amended) The method of claim 41 wherein a nucleic acid selected from the group consisting of (i) a first cancer-associated nucleic acid and (ii) a second cancer-associated nucleic acid, said nucleic acid comprising a metastasis-associated gene, and wherein the presence of said first cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of said second cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.

46. (Amended) The method of either claim 45 or claim 60 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

47. The method of claim 46 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

48. The method of claim 46 wherein the adhesion factor is an adherin.

49. (Amended) The method of either claim 45 or claim 60 wherein the nucleic acid is selected from the group consisting of DNA and RNA.

50. The method of claim 49 wherein the RNA comprises mRNA.

51. The method of claim 50 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

52. (Amended) The method according to any one of claims 41-42 wherein steps (a) - (d) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

53. (Amended) The method according to claim 43 wherein steps (a) - (e) are performed before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell.

54. The method of claim 41 wherein the first nucleic acid is RNA and wherein the second nucleic acid is selected from the group consisting of DNA and RNA.

55. The method of claim 42 wherein the first cancer-specific nucleic acid is RNA and wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA.

56. The method of claim 43 wherein the first cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, wherein the second cancer-specific nucleic acid is selected from the group consisting of DNA and RNA, and wherein the cancer-associated nucleic acid is selected from the group consisting of DNA and RNA.

57. The method of claim 44 wherein the organotypical gene encodes an organotypical marker.

58. The method of claim 44 wherein the first nucleic acid is RNA.

59. The method of claim 58 wherein the RNA comprises mRNA.

60. (New) The method of claim 43 wherein the cancer-associated nucleic acid comprises a metastasis-associated gene, and wherein the presence of the cancer-associated nucleic acid comprising the metastasis-associated gene indicates an increased risk that a disseminated cancer cell has the ability to metastasize, and wherein an increased or decreased presence of the cancer-associated nucleic acid comprising the metastasis-associated gene in said cancer cell relative to the presence or absence of the cancer-associated nucleic acid comprising the metastasis-associated gene in a non-cancer cell from the subject indicates an increased risk that a disseminated cancer cell has the ability to metastasize.